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Dimethylsulfoxide reduction by marine sulfate-reducing bacteria

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Abstract

Dimethylsulfoxide (DMSO) reduction occurred in five out of nine strains of sulfate-reducing bacteria from marine or saline environments, but not in three freshwater isolates. DMSO reduction supported growth in all positive strains. In *Desulfovibrio desulfuricans* strain PA2805, DMSO reduction occurred simultaneously with sulfate reduction and was not effectively inhibited by molybdate, a specific inhibitor of sulfate reduction. The growth yield per mol lactate was 26% higher with DMSO than with sulfate as electron acceptor. In extracts of cells of strain PA2805 grown on sulfate, a low level of DMSO-reducing activity was present (0.013 μmol (mg protein)$^{-1}$ min^{-1}); higher levels were found in cells grown on DMSO (0.56 μmol (mg protein)$^{-1}$ min^{-1}). In anoxic marine environments DMSO reduction by sulfate-reducing bacteria may lead to enhanced dimethylsulfide emission rates.

Keywords: Dimethylsulfoxide reduction; Dimethylsulfide production; Sulfate-reducing bacteria

1. Introduction

Dimethylsulfoxide (DMSO) occurs in freshwater as well as in marine environments [1], in rainwater and in the atmosphere [2]. DMSO arises from the chemical or bacterial oxidation of dimethylsulfide (DMS) [3,4]. DMS plays an important role in the global sulfur cycle and may affect the radiation balance of the earth and thus the climate [5]. DMS also contributes to acid precipitation by its atmospheric oxidation products [6]. It has been found that in marine waters DMSO occurs in higher concentrations than DMS [1]. Oxidation of DMS to DMSO could be a potentially important sink for DMS. DMSO can be reduced to DMS by sulfide in a chemical process [7], or biotically by a wide variety of facultatively anaerobic bacteria which use DMSO as an alternative terminal electron acceptor [8–10].

Previous slurry experiments indicated that DMSO reduction takes place in anoxic marine sediments [11]. Addition of DMSO to marine sediment slurries resulted in the rapid formation of DMS. On the basis of the effect of molybdate, an inhibitor of sulfate reduction, it was concluded that sulfate-reducing bacteria could not be responsible for the observed reduction of DMSO. Because of the availability of DMSO in natural waters and presumably in sediments and the favorable thermodynamics of DMSO reduction compared to sulfate reduction (E_0' $SO_4^{2-}/HS^- = -217$ mV and E_0' DMSO/DMS $= +160$ mV; [12,13]), it would be a relevant strategy for sulfate-

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reducing bacteria to use DMSO as a terminal electron acceptor. For this reason, the capacity of various sulfate-reducing bacteria to use DMSO as a terminal electron acceptor was studied.

2. Materials and methods

2.1. Organisms and cultivation

The following strains were grown in the media as described in the 1993 catalogue of strains from the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig (Germany): *Desulfobacterium (Db.) autotrophicum* DSM 3382 (28°C), *Db. niacini* DSM 2650, *Desulfovibrio (Dv.)* sp. strain HDv DSM 6830, *Dv. salexigens* DSM 2638, *Dv. vulgaris* DSM 1744, *Dv. halophilus* DSM 5663, *Desulfobacter postgatei* DSM 2034 (10 mM acetate). *Dv. desulfuricans* BH (laboratory collection) and *Dv. gigas* NCIMB 9332 were grown in the freshwater medium of Kremer and Hansen [14] and *Desulfovibrio* sp. DSM 3099 in their marine medium. *Desulfobacterium* sp. strain PM4 (laboratory collection) and *Dv. desulfuricans* strain PA2805 were cultivated in the medium described by Heijthuijsen and Hansen [15]. Incubations were at 30°C and the growth substrate was 20 mM lactate, unless otherwise indicated. For all strains, growth and use of DMSO was determined in media with 15 mM sulfate and 5 mM DMSO or 20 mM DMSO as sole electron acceptor. The strains were grown in 120-ml bottles filled with 50 ml medium under an atmosphere of N_2/CO_2 (80%/20%) at pH 7. Abiotic DMSO reduction was determined in non-inoculated medium containing 2 mM sulfide and 20 mM DMSO and amounted 0.05% of the initial DMSO concentration per day.

2.2. Preparation of cell extracts and enzyme measurements

Dv. desulfuricans strain PA2805 was grown to the late exponential growth phase on 20 mM lactate plus 20 mM sulfate or 20 mM DMSO. Cells were harvested by centrifugation (10 min at $11\,000 \times g$) and washed twice with 25 mM potassium phosphate buffer (pH 7.1) containing NaCl (21 g l^{-1}), $MgCl_2 \cdot 6H_2O$ (3 g l^{-1}), and dithiothreitol (2.5 mM). After disrupting the cells twice in a French pressure cell at 140 MPa, the extract was centrifuged (30 min at $40\,000 \times g$). All steps were performed under strictly anaerobic conditions. DMSO and trimethylamine-*N*-oxide (TMAO) reductase activities were measured in anaerobic cuvettes under N_2 at 30°C by following the oxidation of reduced methyl viologen at 578 nm. One unit of enzyme activity corresponds to 2 μmol of methyl viologen oxidized per min. The reaction mixture contained, per ml of 100 mM Tris · HCl buffer (pH 7.8): 250 nmol dithionite, 20 μl cell extract, and 8 μmol methyl viologen. The reaction was started by the addition of 10 μmol DMSO or TMAO.

2.3. Analytical procedures

Lactate and acetate were measured by gas chromatography [15]. See [3] for the analysis of sulfide and sulfate. Protein in cultures was measured by the Lowry method after elemental sulfur extraction with methanol and solubilization of the pellet in 1 M NaOH at 100°C for 10 min; for protein measurement in cell-free extracts the method of Bradford was used. DMS was measured by headspace gas chromatography according to a slight modification of the method of Visscher and van Gemerden [3]: a Supelpak S column was used instead of a Porapak R column. The total amount of DMS per ml of the culture was determined from a calibration curve made with different amounts of DMS in a system with the same gas to liquid ratio, type of medium and temperature as used for the assay of the cultures. DMSO was measured as DMS after reduction with acidified stannous chloride (20 g $SnCl_2 \cdot 2H_2O$ in 100 ml 37% HCl) for 90 min at 55°C.

3. Results

3.1. DMSO reduction by marine sulfate-reducing bacteria

A variety of sulfate-reducing bacteria from freshwater, marine, and hypersaline environments were tested for their ability to reduce DMSO. Four of the marine strains (*Db. niacini*, *Dv. desulfuricans* strain

Table 1
Ability of sulfate-reducing bacteria to reduce DMSO

Species [a]	Environment from which isolate originates	DMSO reduction [b]
Desulfobacterium autotrophicum	Marine	−
Desulfobacterium niacini	Marine	+
Desulfobacterium sp. strain PM4	Marine	−
Desulfovibrio desulfuricans PA2805	Marine	+
Desulfovibrio desulfuricans BH	Fresh	−
Desulfovibrio gigas	Fresh	−
Desulfovibrio salexigens	Marine	−
Desulfovibrio vulgaris	Marine	+
Desulfovibrio halophilus	Hypersaline	+
Desulfovibrio sp. DSM 3099	Marine	+
Desulfovibrio sp. strain HDv	Fresh	−
Desulfobacter postgatei	Brackish	−

[a] For description of strains and growth conditions see Materials and methods.
[b] +, growth and DMSO reduction; −, no growth and no DMSO reduction.

PA2805, *Dv. vulgaris*, *Desulfovibrio* sp. DSM 3099) and one strain from a hypersaline environment (*Dv. halophilus*) were able to grow by DMSO reduction (Table 1). Using a 5% inoculum of sulfate-grown cells, all of these strains stoichiometrically converted 20 mM DMSO to DMS within 5 days of incubation. These strains retained the ability to use DMSO after three successive transfers into fresh medium with DMSO as electron acceptor. None of the other strains were able to reduce DMSO even after 3 weeks incubation. Such negative strains did not reduce DMSO in media with 15 mM sulfate and 5 mM DMSO. The highest level of abiotic DMSO reduction in these cultures was 0.5% of the initial DMSO concentrations per day.

3.2. Characteristics of DMSO reduction by *Desulfovibrio desulfuricans* PA2805

Strain PA2805 was used to study DMSO reduction in more detail. Addition of 10 mM DMSO to an exponentially growing culture in a medium initially containing 20 mM lactate and 20 mM sulfate resulted in an immediate slow production of DMS which rapidly increased (Fig. 1). Sulfate reduction continued after the addition of DMSO; the inoculum

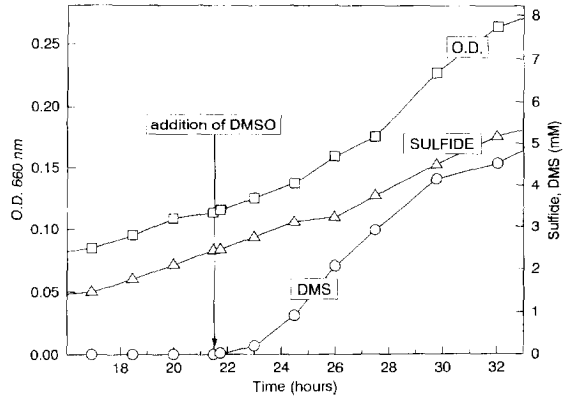

Fig. 1. DMSO reduction by *Desulfovibrio desulfuricans* strain PA2805 after the addition of 10 mM DMSO to a culture initially using 20 mM sulfate. Optical density (O.D.) of culture measured at 660 nm.

came from a culture which had been transferred several times into fresh medium without DMSO.

Addition of 20 mM molybdate and 5 mM DMSO to a culture in the early exponential phase, growing with 20 mM sulfate as electron acceptor, inhibited growth (Fig. 2). However, DMSO was reduced to DMS immediately after its addition. Molybdate interfered with sulfide measurements possibly due to the formation of molybdosulfide complexes. In an abiotic control with 5 mM DMSO, 20 mM molybdate and 5 mM sulfide 0.65 nM DMS was formed per hour.

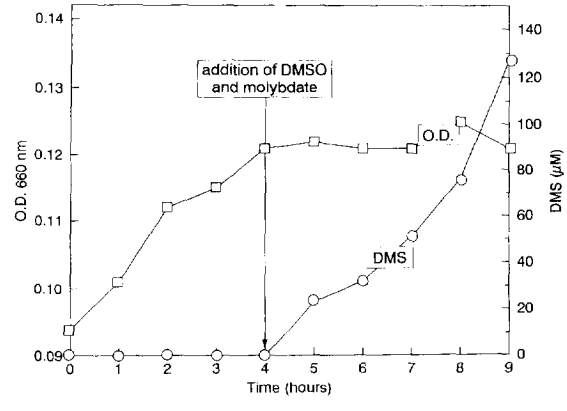

Fig. 2. DMSO reduction by *Desulfovibrio desulfuricans* strain PA2805 after the addition of 5 mM DMSO and 20 mM molybdate to a culture initially growing on 25 mM lactate and 20 mM sulfate. Optical density (O.D.) of culture measured at 660 nm.

Table 2
Growth yield per mol lactate (Y_{mol}), maximum specific growth rate (μ_{max}) and electron donor and acceptor transformation of *Desulfovibrio desulfuricans* strain PA2805 grown on 20 mM lactate plus 20 mM sulfate or 20 mM DMSO

	SO_4^{2-}-grown cultures [a]	DMSO-grown cultures [a]
Lactate utilized (mM)	15.3 ± 1.4	12.6 ± 2.6
SO_4^{2-} utilized (mM)	7.5 ± 1.5	–
Sulfide formed (mM)	5.9 ± 0.7	–
DMSO utilized (mM)	–	24.6 ± 1.2
DMS formed (mM)	–	25.9 ± 2.4
Acetate formed (mM)	13.7 ± 1.4	11.1 ± 2.2
Protein formed (mg l^{-1})	47.8 ± 5.3	49.6 ± 4.3
Y_{mol} (g protein mol^{-1})	3.12 ± 0.06	3.94 ± 0.47
μ_{max} (h^{-1})	0.063 ± 0.009	0.057 ± 0.005
Carbon recovery (%) [b]	99.3 ± 0.8	98.0 ± 1.0
Electron recovery (%) [b]	109.1 ± 1.8	104.0 ± 1.8

[a] Values are the average of two batch cultures.
[b] Carbon and electron recovery was calculated assuming $\langle C_6H_{10.84}N_{1.4}O_{3.07}\rangle$ as the overall composition of cell material [18].

Cell extracts of duplicate cultures of strain PA2805 grown on 20 mM lactate plus 20 mM DMSO had a DMSO reductase activity of 0.56 ± 0.03 units per mg protein. TMAO reductase activity was approx. five-fold the DMSO reductase activity (2.6 ± 0.25 units per mg protein). DMSO and TMAO reductase activities of cell extracts of strain PA2805 grown on 20 mM lactate plus 20 mM sulfate were 0.013 ± 0.001 and 0.095 ± 0.035 units per mg protein, respectively.

The maximum specific growth rates of strain PA2805 with lactate plus sulfate or DMSO as electron acceptor were similar (Table 2). The growth yields per mol lactate plus sulfate or lactate plus DMSO were significantly different, the yield on lactate/DMSO being 26% higher than the yield on lactate/sulfate (Table 2).

4. Discussion

The present results show that some sulfate-reducing bacteria from marine or hypersaline environments are able to grow by the reduction of DMSO and that molybdate does not effectively inhibit DMSO reduction in *Dv. desulfuricans* strain PA2805. Growth inhibition by molybdate is thought to be caused by the depletion of ATP [16]. Under these conditions electron transport from lactate to DMSO might still be possible. The conclusions of a previous study [11] that DMSO reduction does not take place by sulfate-reducing bacteria were based on experiments in which DMSO was added to slurried marine sediments. In these slurries, DMS was produced while sulfate reduction was inhibited with molybdate. Our results imply that sulfate-reducing bacteria still can account for the DMS production in such experiments. Zinder and Brock [9] did not observe DMSO reduction by a freshwater *Desulfovibrio*; we also did not find DMSO reduction by freshwater strains of sulfate-reducing bacteria. However, only a limited number of strains have been tested so far.

In *Dv. desulfuricans* PA2805, DMSO reduction occurred even in the presence of sulfate (Fig. 1); from experiments with media containing 20 mM lactate, 5 mM DMSO and 15 mM sulfate it is concluded that the same was true for the other positive strains, since in all cases the total amount of DMSO was reduced to DMS (data not shown). The ratios between TMAO and DMSO reductase activities of DMSO- or sulfate-grown cells were similar. It is not known whether strain PA2805 contains only one reductase enzyme that is responsible for both DMSO and TMAO reduction as has been established in *Proteus vulgaris*, *Rhodobacter capsulatus* and *R. sphaeroides* [13]. The growth yield per mol lactate of strain PA2805 on DMSO is significantly higher than on sulfate. This indicates that, for sulfate-reducing bacteria, DMSO is potentially a favorable substrate in anoxic marine environments. Sulfate-grown cells still have a low capacity for DMSO reduction; this capacity is strongly enhanced in the presence of DMSO. DMSO measurements in seawater showed up to five-fold higher concentrations of DMSO than of DMS [1]. Unfortunately, no data are presently available on DMSO concentrations in anoxic marine sediments. It can be hypothesized that the DMSO/DMS ratio is high in oxic but low in anoxic environments due to the preferential use of oxygen as terminal electron acceptor in oxic environments. The emission of DMS from these environments to the atmosphere, however, depends on its actual concentration, which is determined by a complex set of

biological and physicochemical processes [17]. In anoxic marine environments where DMSO reduction is readily carried out, a high DMS concentration and thus a high DMS emission can be imagined. DMSO reduction by marine sulfate-reducing bacteria in anoxic marine sediments is probably quantitatively an important process in view of the abundance of these organisms in such environments and the number of species able to reduce DMSO.

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